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Mushroom beta glucan: Potential candidate for post irradiation protection

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ABSTRACT

The in vivo radioprotective effect of a beta-glucan (BG) isolated from the mushroom *Ganoderma lucidum*, against radiation (RT) induced damage was investigated taking mouse survival, hematology, liver GSH (Reduced glutathione), liver Malondialdehyde (MDA) and bone marrow chromosomal aberrations as end points. Young adult swiss albino mice were whole body exposed to gamma radiation. For mouse survival study, BG was administered orally (250 μg/kg body wt or 500 μg/kg body wt) 15 min before or 5 min after 8 Gy exposure. For other parameters BG was given orally 5 min after 4 Gy exposure. The radioprotective effect of BG was compared with that of clinically used radioprotective drug amifostine (WR-2721), at 300 mg/kg body wt administered intraperitoneally, 30 min before irradiation. BG (500 μg/kg body wt) produced (66%) mouse survival at 30 days given post irradiation, and 83% survived at 30 days with 300 mg/kg body wt of amifostine administered before RT while RT alone produced 100% mortality. BG is not toxic at the radioprotective dose. Significant reduction in number of aberrant cells and different types of aberration was observed in both BG and amifostine administered groups compared to radiation alone treated group. BG seems to have potential for use in protection against unplanned radiation exposures.

1. Introduction

Ganoderma lucidum, commonly known as Reishi in Japan and Ling Zhi in China, is well known for its medicinal properties. *G. lucidum* contains a number of compounds among which the polysaccharides and triterpenoids have been identified as the major active components. Crude or partially purified polysaccharides of *G. lucidum* have been reported to inhibit tumor metastasis in mice [1]. The immunomodulating property of this mushroom provides a promising approach for cancer prevention and its administration is found useful alone or in combination with chemotherapy and radiotherapy [1]. Our earlier studies suggest that the aqueous extract of this mushroom has significant radioprotective activity ex vivo [2]. Polysaccharides are among the major source of pharmacologically active constituents of the aqueous extract. Polysaccharides from *G. lucidum* were reported to markedly restore the mitotic activity of bone marrow cells that has been suppressed by antineoplastic drugs [3]. The present study was undertaken to examine the protection offered by beta-glucan, a polysaccharide from *G. lucidum* against radiation induced damage.

2. Materials and methods

2.1. Chemicals

Colchicine was purchased from Sigma Chemicals Co. USA. All other chemicals used in the study were of analytical grade obtained from reputed local manufactures.

2.2. Animals

Swiss albino mice, 6–8 weeks of age and weighing 30 ± 2 g, were selected from the mouse colony of Jawaharlal Nehru Cancer Hospital and Research center, Bhopal, India. They were maintained in air-conditioned animal house and fed on standard mouse food and water ad libitum. Animal handling and experiments were done according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India, and were approved by Institutional Animal Ethics Committee.

2.3. Isolation of beta-glucan

The fruiting bodies of *G. lucidum* were collected from the outskirts of Thrissur district, Kerala, South India. The type specimen was deposited in the herbarium of Center for Advanced Studies in Botany, University of Madras, Chennai, India (HERB. MUBL. 3175). BG was isolated by the method of Mizuno [4] with slight modification [5]. The confirmation of BG was done by Anthrone [6] and phenol sulphuric acid test [7]. Structural confirmation of BG was done by IR and NMR spectrum which were recorded at Sophisticated Analytical Instrument Facility, Indian Institute of Technology, Mumbai, India. The molecular wt of BG was determined by Gel filtration chromatography. The HNMR spectrum suggested that component sugars have beta configuration [5]. From the Gel filtration analysis the molecular weight of BG was found to be 1.5×10^6 Da. The powder was dissolved in double distilled water and administered orally in the experiments.

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2.4. Irradiation

The cobalt therapy unit with Gamma Cell 220 (AECL, Canada) in Jawaharlal Nehru Cancer Hospital, Bhopal was used for irradiation. Unanaesthetized animals were kept in well-ventilated perspex boxes and whole body exposed at a dose rate of 1 Gy/min at a SSD of 99.67 cm.

2.5. Survival assay

The animals were divided into 7 groups of 10 animals each and treated as follows

Group I – Control [Double distilled water (DDW)]
Group II – DDW (5 min before irradiation) + Radiation 8 Gy
Group III – Amifostine (300 mg/kg body wt) 30 min before irradiation + Radiation 8 Gy
Group IV – Radiation 8 Gy + BG 250 μg/kg body wt (5 min after irradiation)
Group V – Radiation 8 Gy + BG 500 μg/kg body wt (5 min after irradiation)
Group VI – BG 250 μg/kg body wt (15 min before irradiation) + Radiation 8 Gy
Group V II – BG 500 μg/kg body wt (15 min before irradiation) + Radiation 8 Gy

Animals were observed for survival up to 30 days post irradiation. Data presented are % survival on 30 days.

2.6. Hematology

Five groups with 15 animals each were treated as follows
Group I – Control (DDW)
Group II – DDW (5 min before irradiation) + Radiation 4 Gy
Group III – Amifostine (300 mg/kg body wt) 30 min before irradiation + Radiation 4 Gy
Group IV – Radiation 4 Gy + BG (250 μg/kg body wt) (5 min after irradiation)
Group V – Radiation 4 Gy + BG (500 μg/kg body wt) (5 min after irradiation)

BG was administered 5 min after irradiation and amifostine was administered 30 min before irradiation. 3 mice from each group were sacrificed by cervical dislocation on days 1, 3, 5, 7 and 9 post treatment and blood was collected from heart of the animal for hematological studies and the liver after removing gall bladder was stored on ice for biochemical studies.

Hemoglobin was estimated by the method of Drabkin and Austin [8]. The reagent used was from Agappe diagnostic kit. 0.02 ml fresh whole blood was mixed with 5 ml of cyanmeth reagent. The OD was measured at 546 nm by the use of a Varian DMS 200 UV-visible spectrophotometer against reagent blank after 5 min incubation at room temperature. Total leucocytes were studied according to the method of Chaudari [9]. For this 0.02 ml blood was added to 0.38 ml of diluting fluid and charged the Neubauer counting chamber with the well mixed diluted blood, WBC cells were counted in 4 large corner squares of chamber after 3–4 min. Total number of WBC = No of Cells counted × 50, expressed as counts/mm^3.

2.7. Liver biochemistry

2.7.1. GSH

Reduced glutathione (GSH) in the liver of mice was determined by the method of Moron et al. [10]. 0.5 ml of tissue homogenate was mixed with 0.1 ml of 25% TCA (trichloro acetic acid) and kept on ice for 5 min and then subjected to centrifugation at 3000 rpm for 10 min to settle the precipitate. 0.3 ml of the supernatant was mixed with 0.7 ml of 0.2 M sodium phosphate buffer (pH 8). The yellow color obtained was measured after 10 min at 412 nm by the use of a Varian DMS 200 UV-visible spectrophotometer against a blank which contained 0.1 ml of 5% TCA in place of the supernatant. The GSH content was calculated with the help of this standard graph and expressed as μ/mg protein.

2.7.2. MDA

The level of lipid peroxidation as malondialdehyde (MDA) in liver of mice was determined according to the method of Ohkawa et al. [11]. 4 ml of reaction mixture containing 0.4 ml of tissue homogenate, 1.5 ml of 0.8% TBA (thio barbituric acid), 1.5 ml acetic acid (20%, pH 3.5) and distilled water was kept for 1 h in a boiling water bath at 95 °C. After 1 h the reaction mixture was removed from the water bath, cooled and added 1 ml of distilled water. 5 ml of butanol:pyridine mixture (3:1) was added to the reaction mixture mixed thoroughly and centrifuged at 3000 rpm for 10 min. Absorbance of clear supernatant was measured at 532 nm by the use of a Varian DMS 200 UV-visible spectrophotometer against butanol:pyridine mixture. The MDA was calculated with help of standard graph made with 1′1′3′3′-tetra methoxy propane in 1 ml distilled water and is expressed as μ/mg protein.

2.8. Chromosomal aberrations

The animals were divided into 6 groups of 6 animals each and treated as follows

Group I – Control (DDW)
Group II – Amifostine (300 mg/kg body wt)
Group III – BG (500 μg/kg body wt)
Group IV – DDW (5 min before irradiation) + Radiation 4 Gy
Group V – Amifostine (30 min before irradiation) + Radiation 4 Gy
Group VI – Radiation 4 Gy + 500 BG (500 μg/kg body wt) (5 min after irradiation)

2.8.1. Metaphase preparation

At 22 h after irradiation all the animals were injected i.p. with 0.025% colchicine and sacrificed 2 h later by cervical dislocation. Both femurs were dissected out and metaphase plates were prepared by air-drying method [12]. Briefly, bone marrow from the femur was aspirated, washed in saline, treated hypotonically (0.565% KCl), fixed in 3:1: methanol:acetic acid, spread on clean slides and stained with 4% Giemsa. Chromosomal aberrations were scored under light microscope. A total of 500 metaphases were scored per animal. Different types of aberrations like chromatid breaks, chromosome breaks, fragments, rings, dicentrics, severely damaged cells (SDC, cells with 10 or more aberrations of any type) and cells showing polyploidy were scored. When breaks involved both the chromatids it was termed as "chromosome type" aberration, while "chromatid type" aberration involved only 1 chromatid. If the deleted portion had no apparent relation to a specific chromosome, it was called a fragment [13]. Data are presented as mean ± S.E.

2.9. Ferric reducing antioxidant power (FRAP) assay

The ferric reducing ability was measured at low pH [14,15]. The stock solution of 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ), 40 mM HCl, 20 mM $FeCl_3 \cdot 6H_2O$, and 0.3 M acetate buffer (pH 3.6) were prepared. The FRAP reagent was mixed with 90 μl water and 30 μl BG/distilled water/standard antioxidant solution. The reaction mixture was then incubated at 37 °C for 30 min, and absorbance was recorded at 595 nm. An intense blue color complex was formed when ferric tripyridyl triazine (Fe^{3+} TPTZ) complex was reduced to the ferrous (Fe^{2+}) form and the absorbtion at 595 nm was recorded. The calibration curve was plotted with absorbance at 595 nm versus concentration of $FeSO_4$ which in turn plotted against concentrations of standard antioxidant (ascorbic acid). The absorbance were recorded by the use of a Varian DMS 200 UV-visible spectrophotometer.

2.10. Inhibition of lipid peroxidation

The reaction mixture contained 0.1 ml of rat liver homogenate (25%, w/v) in Tris–HCl buffer (20 mM, pH 7), $FeSO_4$ $(NH_4)_2SO_4 \cdot 6H_2O$ (0.16 mM), ascorbate (0.06 mM) and various concentrations of BG in a final volume of 0.5 ml [11]. The reaction mixture was incubated for 1 h at 37 °C. After the incubation period, 0.4 ml was removed and treated with 0.2 ml SDS (8.1%), 1.5 ml thiobarbituric acid (0.8%) and 1.5 ml acetic acid (20%, pH 3.5). The total volume was made up to 4 ml with distilled water and then kept in a water bath at 95–100 °C for 1 h. After cooling, 1 ml of distilled water and 5 ml of n-butanol and pyridine mixture (15:1) were added to the reaction mixture, shaken vigorously and centrifuged at 4000 rpm for 10 min. The n-butanol–pyridine layer was removed and its absorbance at 532 nm was read spectrophotometrically. Inhibition of lipid per oxidation was determined by comparing the optical density of the treatments with that of control.

2.11. Hydroxyl radical scavenging activity

The reaction mixture contained deoxyribose (2.8 mM), $FeCl_3$ (0.1 mM), KH_2PO_4–KOH buffer (20 mM, pH 7.4), EDTA (0.1 mM), H_2O_2 (1 mM), ascorbic acid (0.1 mM) and various concentration of BG in a final volume of 1 ml. The reaction mixture was incubated at 37 °C for 1 h. The TBARS formed was estimated by thiobarbituric acid method of Ohkawa et al. [11]. The hydroxyl radical scavenging activity was determined by comparing absorbance of control with that of treatments. The absorbance was recorded spectrophotometrically.

2.12. Statistical analysis

Data were analyzed by Student's *t*-test. A value of $P < 0.05$ was considered to be significant.

3. Results

3.1. Survival

80% of animals died within 20 days after exposure to 8 Gy gamma irradiation in the radiation alone treated group. No mice survived on 30th day in this group (Fig. 1). Administration of BG alone at 500 μg/kg body wt and amifostine alone at 300 mg/kg body wt did not produce any toxicity and showed 100% survival. Pre irradiation administration of BG at 250 μg/kg body wt and 500 μg/kg body wt showed 50% and 66% survival at 20th day and